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Studies on the Genetics of Smuts of Barley and Oats in Relation to Pathogenicity

Clyde C. Allison
Division of Plant Pathology and Botany

University of Minnesota
Agricultural Experiment Station

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STUDIES ON THE GENETICS OF SMUTS OF BARLEY AND OATS IN RELATION TO PATHOGENICITY

CLYDE C. ALLISON[1]

INTRODUCTION

Smuts of barley occur wherever barley is grown. In some areas loose smut caused by *Ustilago nuda* (Jens.) K. and S. is more prevalent than covered smut, *Ustilago hordei* (Pers.) K. and S., while in other areas the situation may be the reverse (25). In Minnesota *U. nuda* is more prevalent than *U. hordei.* Two factors probably account for the distribution of these two smuts in Minnesota. Of primary importance is the susceptibility of most of the commonly grown varieties to loose smut, while only one variety, Trebi, is highly susceptible to *U. hordei,* according to field observations. Secondly, the inconvenient and somewhat unreliable method of seed treatment to control loose smut may contribute to its prevalence, whereas covered smut is easily controlled by treating the seed with fungicides. Nevertheless, *U. hordei* often causes considerable damage in the state. Fields of Trebi barley averaging 5 to 10 per cent of smutted heads are not uncommon, and some fields of barley have been observed with 50 per cent of smutted heads. The smut reactions of some of the more recently introduced varieties and new hybrid lines are not known except through general observations in commercial fields and experimental field plots.

One of the greatest handicaps in varietal testing, or other investigations involving inoculation with covered smut of barley, has been the low percentage of smutted heads resulting from artificial inoculation with chlamydospores. Previous workers have overcome this to a certain extent by dehulling the seeds prior to inoculation (24, 25, 39, 40), but this method is laborious and time-consuming and sometimes lowers the percentage of seed germination. No method of inoculating seed of barley or oats with sporidial lines has proved reliable and practical on a large scale. Different methods have been used with varying results by different workers, but most of them require too much time and labor to justify their use.

One of the first steps, then, in studying the pathogenicity and genetics of *U. hordei* was to devise a reliable and simplified method of inoculation. With the method developed by Haarring (16) and Zade (43) in inoculating oat seeds with chlamydospores of *Ustilago avenae* (Pers.) Jens., as a basis, a satisfactory method of inoculating oat and barley

[1] The writer acknowledges with pleasure his gratitude and indebtedness to Dr. E. C. Stakman for suggesting this problem, for his inspiration, and for many valuable criticisms during the progress of the investigation and in writing the manuscript. Thanks are due also to Dr. J. J. Christensen for many valuable suggestions for the advancement of this investigation and for his invaluable encouragement. Further obligations are acknowledged to Dr. E. L. LeClerg for valuable aid in the statistical analysis of the data, to all the investigators who collected barley smut specimens, and to Dr. Helen Hart for help in preparation of the manuscript.

seeds with sporidial lines of chlamydospores was devised and used in the present investigations.

Altho covered smut of barley is readily controlled by seed treatment, the use of resistant varieties would be more desirable. It is known, however, that some varieties of crop plants do not always remain resistant to certain diseases over a period of years (20, 36). In some cases this is due to the origin or introduction of new parasitic races of the pathogen and not to change in the resistance of the variety. This naturally increases the difficulty of obtaining and maintaining resistant varieties. Faris (13), in 1924, and Rodenhiser (34), in 1928, demonstrated parasitic races within *U. hordei.* However, very little is known regarding their distribution or virulence on varieties of barley grown in Minnesota. Obviously this information would be useful in a barley improvement program.

Biedenkopf (3), in 1894, described an intermediate type of barley smut as *Ustilago medians,* altho Brefeld (4) did not consider it sufficiently distinct from *U. nuda* to justify recognizing it as a new species. No further reports of such an intermediate type were published until 1932, when Tapke (38) described a new loose smut which he called *Ustilago nigra.* In the fall of 1932 Dr. J. J. Christensen collected several heads of smut on Minsturdi barley in the plant pathology plots at University Farm, St. Paul, Minnesota, which appeared similar to *U. medians* and gave them to the writer for further investigation. Subsequently, certain other collections of barley smut examined by the writer were found to be intermediate between *U. hordei* and *U. nuda.*

Whether the intermediate types of barley smut are important depends on their distribution, their pathogenicity on commonly grown varieties, the ease with which they hybridize with *U. hordei, U. nuda,* and each other, and the pathogenicity and mode of infection of the hybrids. The writer, therefore, made a study of the possible results of hybridization.

Kniep (28) showed that sporidia and promycelial cells of different species of Ustilago, including *U. hordei* and *U. levis,* might fuse and produce dicaryophytes in which the conjugate nuclei were derived from different species. This was later confirmed by Dickinson (10). Neither of these investigators, however, demonstrated the pathogenicity of interspecific sporidial combinations. It therefore seemed very probable that *U. hordei* might hybridize freely with *U. medians,* and possibly with certain other cereal smuts.

MATERIALS AND METHODS

The barley and oat seed used was obtained from the Division of Agronomy and Plant Genetics, University Farm, St. Paul, Minnesota. Prior to inoculation, all seed was soaked for 13 minutes in water at 52° C., to free it from natural smut infection.

The sources of chlamydospore collections are indicated in Table 1. The head type was described when the collection was received and spore-wall markings were observed under water or oil immersion lenses.

All sporidial lines used were derived from single sporidia isolated with a Zeiss micromanipulator, according to the method described by

Table 1. Source and Character of Barley Smut Collections

Collection number	Date collected	Collector	Vicinity	Head type	Character of spore wall
1	1932	Allison and Graham	Osseo, Minnesota	Compact	Smooth
5	do	W. Butler	Chadron, Nebraska	Loose	Smooth and echinulate
11	do	Allison and Graham	Osseo, Minnesota	Compact	Smooth
12	do	W. Butler	Pierce, Nebraska	do	do
14	do	C. C. Allison	Armstrong, Iowa	Loose	Echinulate
15	do	do	Northwood, Iowa	Compact	Smooth
16	do	E. C. Stakman	Lake City, Minnesota	do	do
17	do	L. W. Melander	Martin Co., Minnesota	do	Smooth and echinulate
18	do	C. C. Allison	Madelia, Minnesota	do	Smooth
19	do	W. Butler	Chadron, Nebraska	do	do
20	do	C. C. Allison	Armstrong, Iowa	do	do
21	do	W. Butler	Lexington, Missouri	do	do
22	do	do	Stockton, Kansas	do	do
23	do	J. J. Christensen	Stewart, Minnesota	do	do
26	do	C. C. Allison	Champlin, Minnesota	do	do
27	do	J. J. Christensen	St. Paul, Minnesota	Loose	Echinulate
28	do	W. Butler	Lowry, South Dakota	Compact	Smooth
29	do	J. J. Christensen	Sumter, Minnesota	do	do
30	do	C. C. Allison	Zimmerman, Iowa	Intermediate	do
31	1933	W. Butler	Humphrey, Nebraska	Compact	do
32	.....	.....	Unknown	Loose	do
33	1932	E. C. Stakman	Edmonds, North Dakota	Intermediate	Smooth and echinulate
34	do	Geo. Hafstad and J. G. Churchward	Lewiston, Idaho	Compact	Smooth
36	do	M. B. Moore	Porter, Minnesota	do	do
37	1930	C. C. Allison	Kenyon, Minnesota	do	do
38	1931	R. C. Rose	St. Peter, Minnesota	do	do
39	do	do	Echo, Minnesota	do	do
40	do	do	do	do	do
41	do	C. C. Allison	Fertile, Minnesota	do	do
42	do	C. J. Eide	St. Paul, Minnesota	do	do
43	do	W. Butler	Exp. Sta., Mexico City	do	do
46	do	do	do	do	do
47	do	do	do	do	do

Hanna (17). Each chlamydospore was given a number or letter, and the sporidia were numbered according to their position on the promycelium, beginning at the apex. In most cases the collection number also was used. Thus, culture 17-A-1 originated from the sporidium taken from promycelial cell number 1, chlamydospore A, collection number 17. Later, for convenience, all sporidial lines except certain f_2 lines[2] were given a single number; for example, *U. medians* culture 24.

Potato-dextrose agar, consisting of the extract of 50 grams of potatoes, 0.5 per cent dextrose, and 1.5 per cent agar, was used in studying the cultural characters of the various sporidial lines. Slightly alkaline 1.5 per cent plain agar was most suitable for studying sexual fusions between sporidia of different monosporidial lines. Three-day-old cultures of different sporidial lines were combined on this agar in petri dishes and observed for presence or absence of fusions after 18 hours.

In preparing slides to study the nuclear conditions of fused sporidia, egg albumen was used as a fixative. After applying the fixative, the slide was covered with a thin layer of 1.5 per cent plain agar. The sporidial lines used were then transferred to the slide, excellent mixture and distribution resulting when a drop of water was added to the inoculum. The slides were then inverted over glass rods in moist petri dishes until the fusions had developed to the desired stage, when the material was killed with Fleming's weaker solution. The method of staining was similar to Yamanouchi's schedule, as given by Chamberlain (6), for using Haidenhain's iron-alum haematoxylin.

All inoculations, except where otherwise stated, were made by means of the partial vacuum method described later. In the case of sporidial inoculations, monosporidial lines were grown singly in a potato dextrose solution (0.5 per cent dextrose) in test tubes or in flasks. The desired combinations of lines were then made in separate tubes or flasks to which seed was added and then evacuated, dried 12 hours, and sown or stored at 2° C.

EXPERIMENTAL RESULTS

Effectiveness of Inoculation Methods

Attempts to produce good infection by dusting barley seed with chlamydospores of *U. hordei* have not been satisfactory, altho this method was more satisfactory for inoculating oats with *U. levis* and *U. avenae* chlamydospores.

Jensen (24), in 1888, pointed out that infection of barley and oat seedlings seldom resulted from spores adhering to the seed. He did, however, obtain satisfactory evidence that when dehulled seeds of barley and oats were dusted with chlamydospores about 25 per cent of the plants produced were smutted. He concluded that in nature chlamydospores lodged underneath the hulls were responsible for infection. Tisdale (39), in 1923, emphasized the importance of devising a satisfactory method of artificial inoculation of barley seed with *U. hordei.*

[2] Gametic filial generations are designated by f and chlamydospore generations by the usual F.

Briggs (5), in 1927, demonstrated that barley seeds dehulled by sulphuric acid and inoculated with covered smut produced about the same percentage of smutted plants as seed dehulled by hand, but the acid caused considerable seed injury, and it was later found that the concentration and duration of treatment necessary to dehull the seed may differ for different varieties.

The most recent improvement in inoculating seeds of cereals artificially was made by Zade (43) and later improved by Haarring (16). Haarring placed oat seeds in a chlamydospore suspension in nutrient solution and evacuated the seeds in partial vacuum until no more air bubbles arose from them. He then dried the seed for 24 hours at a temperature of 18 to 20° C. The seed was then placed on moistened filter paper in a saturated atmosphere. After 20 hours the seed was allowed to dry slowly for two days and then sown.

The work of Zade and of Haarring suggested the use of the partial vacuum method for the inoculation of barley with chlamydospores of *U. hordei.* Preliminary experiments by the writer in the greenhouse indicated that the vacuum method was considerably better than dusting and required less time than dehulling and dusting the seeds. Accordingly, more extensive experiments were planned to determine the effectiveness and reliability of the partial vacuum method. Approximately 100 seeds of each of five varieties of barley were inoculated in duplicate by three different methods with chlamydospores of *U. hordei.* To one series were added sufficient dry chlamydospores to cover the seeds after being well shaken. The other two series were inoculated by modifications of the method used by Haarring. Approximately 100 seeds of each variety were placed in a test tube to which was added 10 cubic centimeters of a suspension of chlamydospores. Four tubes of each variety were made up in this manner, placed in a desiccator attached to a motor vacuum pump, and evacuated for 20 minutes. The seed was then allowed to dry for 12 hours, after which two lots of each variety were placed on filter paper in a petri dish to which 2 cubic centimeters of water had been added. After 24 hours the seed was sown while still moist. The other two lots of seed of each variety, after drying 12 hours, were stored at 2° C. for 24 hours. All seed was sown in five-foot rows on May 21, 1932. The results are given in Table 2.

Except in the case of Trebi barley, the percentage of smutted heads was low, but more smut developed in all varieties inoculated by the vacuum methods than when the seed was simply dusted. The number of smutted heads produced from seed inoculated by the two partial vacuum methods differed but little, and the second was adopted because of its simplicity.

In further tests five varieties were inoculated with chlamydospores of five collections of smut, using the partial vacuum and dusting methods. The percentages of smutted heads resulting from the use of the partial vacuum method are given in Table 3. The percentages of smutted heads in plants from chlamydospore-dusted seed are not given, since less than two per cent of the heads in any row were smutted. The results of these two experiments indicated definitely that the partial vacuum method was far superior to the dusting method.

Table 2. Percentage of Covered Smut Obtained on Five Varieties of Barley by Inoculating the Seed with Chlamydospores by the Vacuum and by the Dusting Methods

Method of inoculation	Manchuria	Peatland	Svansota	Trebi	Velvet
Vacuum*					
Number heads	230	291	213	207	233
Number smutted	8	8	9	57	1
Per cent smut	3.5	2.8	4.2	27.5	0.4
Vacuum†					
Number heads	218	253	224	192	205
Number smutted	9	6	11	49	3
Per cent smut	4.1	2.4	4.9	25.5	1.5
Dust					
Number heads	142	111	116	137	119
Number smutted	0	0	3	6	0
Per cent smut	0	0	2.6	4.4	0

* Seed evacuated, dried 12 hours, moistened 24 hours, and sown.
† Seed evacuated, dried 12 hours, and stored at 2° C. for 24 hours.

The partial vacuum method appeared to solve another problem of inoculation, i.e., the inoculation of barley and oat seed with monosporidial lines. This method was tried and found entirely satisfactory, and it was therefore adopted for all sporidial inoculations.

Physiologic Specialization

It is difficult to say just what constitutes a physiologic race in cereal smuts. As a rule, investigators have referred to a collection of smut with distinctive pathogenicity as a physiologic race. Since collections of smut chlamydospores usually comprise numerous biotypes, or at least potential biotypes, there may be some objection to calling them physiologic races. However, they are so designated in this paper, as a matter of convenience.

Faris (13), in 1924, demonstrated physiologic races within *U. hordei.* He used three spring varieties and one winter variety of barley as differential hosts on which he distinguished five physiologic races. Rodenhiser (34) distinguished seven cultural races of *U. hordei,* two of which differed in pathogenicity on Lion and Himalaya barley. Race 1, from Italy, produced 7.2 per cent smut on Lion but no smut on Himalaya. Race 2 produced 17.0 per cent smut on Himalaya and no smut on Lion. On the other varieties used, Manchuria, Svansota, Trebi, and Wisconsin Pedigree, only a trace of smut was produced by either race.

As the physiologic races of *U. hordei* demonstrated by Faris and by Rodenhiser were not differentiated on varieties of barley commonly grown in Minnesota and surrounding states, it seemed advisable to determine the reaction of the commonly grown varieties of barley to collections of barley smut from this region and others from which smut is likely to be introduced. Accordingly, in 1933, the pathogenicity of five collections of *U. hordei* was determined on five barley varieties.

The results are given in Table 3. Four of the collections appeared to be nearly identical in their effects on the five varieties. Collection 38, however, appeared to be less virulent on all varieties except Glabron.

Table 3. Reaction of Five Barley Varieties to Five Collections of Ustilago hordei

Collection	Variety and percentage of smutted heads*				
	Glabron	Peatland	Svansota	Trebi	Velvet
38	2	4	0	10	4
39	4	10	8	30	14
40	4	10	10	31	10
42	0	8	11	29	12
43	6	13	10	27	8

* Percentage computed on total number of plants in duplicate five-foot rows.

In 1934 the work was expanded, and 15 varieties and lines of barley were inoculated with various collections of barley smuts. Four of the varieties used are omitted from Table 4, since the strain of Manchuria was not known and the other three varieties were highly resistant and not widely grown in Minnesota and neighboring states. The source and character of the barley smut collections are given in Table 1.

The percentages of smut in duplicate rows of each variety are given in Table 4. These data were analyzed by Fisher's analysis of variance method (14). From Table 5 it appears that there are highly significant differences between collections and between varieties and in the interaction of collections with varieties since the value of Z greatly exceeds the one per cent point. Taking twice the standard error of the differences between any two means ($3.76 = 7.5$) as the minimum level of significance, it is apparent that there are distinct differences in the reactions of some of the barley varieties to the different smut collections.

Trebi and Minnesota No. 474 were, in general, the most susceptible varieties used. Certain collections did not attack one or the other of these varieties. Collection 33 produced no smut on Minnesota No. 474, but caused 19 per cent of smutted heads in Trebi. The reverse was true with collections 46 and 47, which produced 27 and 36 per cent of smutted heads, respectively, in Minnesota 474, but none in Trebi. Four varieties, Svansota, Trebi, Minsturdi, and Minnesota No. 474, appeared to be excellent differential varieties. Certain collections could be differentiated also on Velvet, Peatland, Odessa, Oderbrucker, and Minnesota No. 462. Glabron and Wisconsin No. 38 were highly resistant to all of the collections used. Collections 11 and 26, 28 and 29, and 12 and 43 could not be differentiated on Svansota, Trebi, Minsturdi, or Minnesota No. 474. However, collections 11 and 26 could be differentiated on Peatland, and collections 28 and 29 on Minnesota No. 462. Collections 12 and 43 were identical in their effects on all the varieties used, but were the only two collections that behaved exactly alike. In other words, according to the statistical analysis of these data, 27 of the 28 collections may be differentiated on six varieties.

Table 4. Reaction of Barley Varieties to 28 Smut Collections

Collection	Variety and percentage of smutted heads										
	Velvet	Glabron	Svansota	Peatland	Trebi	Odessa	Wis. No. 38	Min-sturdi	Oder-brucker	Minn. No. 474	Minn. No. 462
1	9	0	14	1	16	11	2	10	7	29	5
11	1	1	3	1	15	3	0	9	8	19	5
12	8	3	12	1	15	6	0	10	3	11	8
16	1	0	2	4	9	2	1	17	8	15	0
17	8	0	14	11	15	3	0	20	6	21	4
18	9	2	11	3	10	12	3	16	2	20	9
19	0	1	0	0	11	4	0	3	1	11	0
20	4	0	1	0	7	3	0	8	0	5	7
21	2	1	13	1	10	7	0	3	2	15	4
22	0	0	11	0	12	7	0	0	0	3	0
23	9	2	3	2	23	7	2	17	7	11	6
26	6	3	3	9	15	4	2	13	11	15	2
28	2	0	4	2	14	4	1	9	0	17	2
29	6	0	2	2	11	7	0	13	3	21	10
30	9	2	7	5	15	5	0	19	5	30	8
31	5	3	1	0	17	6	0	19	6	27	3
32	7	0	7	7	15	7	3	15	4	20	4
33	0	0	1	1	19	4	1	1	2	0	0
36	5	1	3	1	15	2	0	12	3	8	6
37	1	1	1	2	11	6	1	7	7	33	6
38	3	0	3	4	8	3	1	12	5	13	8
39	8	1	3	4	8	10	1	16	9	24	6
40	3	0	8	2	16	4	1	15	6	23	8
41	1	1	16	4	14	8	0	9	5	18	4
42	4	0	6	0	10	6	0	16	11	11	10
43	3	1	13	1	15	6	2	11	3	14	8
46	0	2	0	0	0	0	0	0	0	27	0
47	0	1	0	0	0	0	0	0	0	36	2

Table 5. The Analysis of Variance of Reaction of Barley Varieties to Various Smut Collections

Variations due to	D.F.	Sum of squares	Variance	Z
Replications	1	5.77	5.77	
Collections	27	3,172.04	117.48	1.0589**
Varieties	10	14,943.12	1,494.31	2.3308**
Collections x Varieties	270	9,972.98	36.94	0.4805**
Error	307	4,336.57	14.13	
Total	615	32,430.48		

** Value of Z exceeds 1 per cent point.
S.E. of difference between two means = 3.76.

The writer hesitates to consider all of these collections as distinct parasitic races. Altho the 28 collections behaved like distinct parasitic races in these tests, it is possible that their behavior would not be entirely consistent. It will be noted that the five collections used in 1932 did not have the same pathogenicity on the same varieties in the 1933 tests. The presence of different biotypes within the collections might account for the change in pathogenicity, since certain biotypes might predominate one year whereas other biotypes might be more prevalent in other years. In addition, if the collections do consist of different biotypes, then new biotypes could readily be produced by hybridization or mutation. Nevertheless some of the 28 collections differed from one another so definitely that they must be considered distinct parasitic races, and no doubt play an important role in the degree of susceptibility of our commonly grown barley varieties.

Further evidence of the existence of different parasitic races was obtained when seed of Minnesota No. 474 was inoculated with chlamydospore collections 31, 33, and 47 and sown in the greenhouse. Collection 33 produced no smut on Minnesota No. 474 in the field, but caused smut on plants of that variety in the greenhouse. However, it did not produce normal smutted heads, but caused the formation of smut galls in place of a smutted head (Fig. 1). Collection 46, to which Minnesota No. 474 was very susceptible in the field, produced typical smutted heads on that variety in the greenhouse. In the field 27 per cent of the heads of Minnesota No. 474 were smutted by collection 31, and normal smutted heads were produced. In the greenhouse Minnesota No. 474 was again susceptible to collection 31, but the smutted heads usually did not emerge from the sheath, and the sheath and upper leaves were badly smutted (Fig. 5). This characteristic smutting of plants of Minnesota No. 474 in the greenhouse by the three smut collections, 31, 33, and 47, was very distinct and general on all plants smutted by each collection. This indicates then, at least with Minnesota No. 474, that collections of smut may be differentiated on the basis of the type of smutted heads or culms produced when the plants are grown under certain environmental conditions. The explanation for these different types of smutted plants must certainly depend on the host-parasite relationship, but just what this relationship is has not been determined.

In 1934 seed of the varieties used in 1933 was inoculated with the same smut collections, three new collections of *U. medians,* and five addi-

tional collections of *U. hordei.* The seed was sown in triplicate rows, but because of the extremely dry weather the results were inconclusive and are not given here.

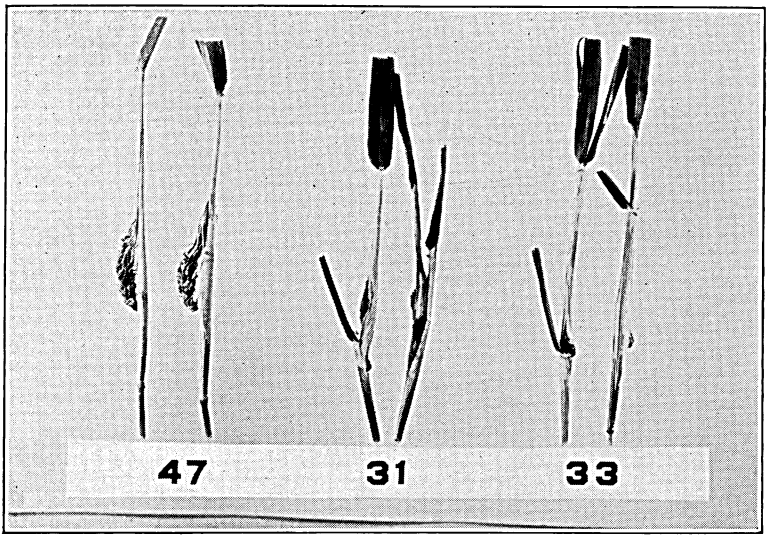

FIG. 1. SMUTTED PLANTS OF MINN. NO. 474 PRODUCED FROM SEED INOCULATED WITH CHLAMYDOSPORES OF *U. hordei* COLLECTIONS 47, 31, AND 33 AND GROWN IN THE GREENHOUSE

Segregation of Factors for Sex and Cultural Characters

Dickinson (10) found that the segregation for sex factors in *U. hordei* was in the ratio of 2:2. Hanna and Popp (19) and Holton (21) obtained the same results for *U. avenae* and *U. levis.* In other smuts ratios of 4:0, 3:1, and 2:2 have been observed (2, 8, 12, 18, 35, 37). Dickinson (10) and Holton (22) have demonstrated that there were six possible arrangements of the sex groups on the promycelium of *U. levis.*

In studying the nature of segregation within *Ustilago hordei* and *U. levis* Dickinson found that factors for cultural characters segregated in 2:2, 3:1, and 4:0 ratios and that segregation for cultural characters was independent of segregation of sex factors. Other workers have found the same to be true in other smut fungi (8, 18, 35, 38).

In order to determine the segregation of factors for sex and cultural characters, the writer isolated complete sets of four sporidia from 20 germinating chlamydospores of *U. hordei.* Each set of four sporidial lines was paired in all possible combinations, and combinations between sets also were made. On the basis of sporidial fusions, there were six possible arrangements of the + and — sporidia on the promycelia. (See Table 6.)

Table 6. Possible Arrangements of + and — Sporidia on Promycelia of Ustilago hordei, Each Vertical Column Representing a Promycelium

Segregation of sex factors	
Reduction in first division	Reduction in second division
+ —	+ — + —
+ —	— + — +
— +	+ — — +
— +	— + + —

The monosporidial lines were then grown on potato dextrose agar in flasks under identical laboratory conditions to determine the cultural characters of the different sporidial lines. It was found that segregation of factors for cultural characters did not necessarily occur at the same time as segregation of sex factors. The only segregation ratio obtained for sex factors was 2:2, while 3:1, 2:2, and 0:4 ratios were obtained for different cultural characters, such as color, topography, consistency, and margin of colony. In some cases the two sporidial lines of the same sex were similar in cultural characters, while in other cases they were entirely different. The results agree with those of others who found two sex groups in *U. hordei* arranged on the promycelium according to chance. The fact that the factors for cultural characters may segregate independently of the factors for sex agrees with the results obtained by other workers with *U. hordei* and other smuts.

Four complete sets of sporidia of *U. medians* also were paired in all possible combinations. There were two sex groups and at least two arrangements of the + and — sporidia on the promycelium. If a larger number of complete sets of sporidia had been studied, it is probable that the six possible arrangements of the + and — sporidia could have been demonstrated.

Mutations in *Ustilago bromivora* (Tul.) F. V. Waldh. were observed by Bauch (2) in 1925. Since then it has been demonstrated that mutations occur very commonly in many smut fungi (8, 9, 18, 22, 34, 38). Rodenhiser (34) reports the appearance of wedge-shaped sectors in cultures of *U. hordei,* but, owing to the fact that the original isolations were made from a mass chlamydospore collection, he concluded that these sectors may or may not have been true mutants.

The writer observed sectors in many of the monosporidial lines of *U. hordei* and *U. medians* studied in culture and is of the opinion that these sectors were the result of mutation rather than delayed segregation. Single sporidia were isolated from many of the sectors and the mutants were cultured and compared with the parent monosporidial lines in cultural characters. The mutants differed from the parent colonies in rate, color, and type of growth. As a rule the mutants were more myceloid than the parents. Photographs of two parent cultures of *U. hordei* and their respective mutants are shown in Figures 2 and 3. The two mutants were identical, altho the parent monosporidial lines came from two chlamydospores from different collections.

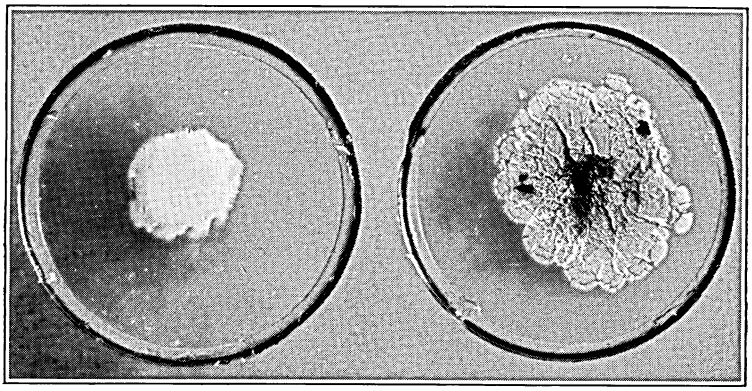

FIG. 2. CULTURAL CHARACTER OF *U. hordei* GROWN ON POTATO-DEXTROSE AGAR
Left: Monosporidial Culture 46 Right: Mutant from Culture 46

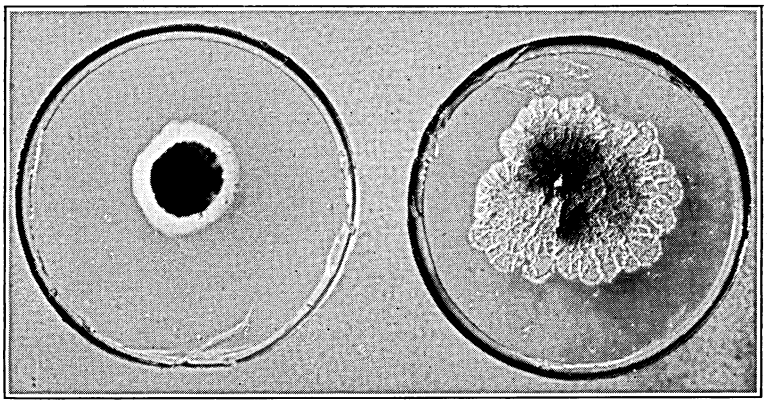

FIG. 3. CULTURAL CHARACTER OF *U. hordei* GROWN ON POTATO-DEXTROSE AGAR
Left: Monosporidial Culture 12 Right: Mutant from Culture 12

Late in the spring of 1933, in an effort to determine if the pathogenic capabilities of the mutants differed from those of the parent lines, five barley varieties were inoculated with different compatible combinations of mutants x mutants, parent lines x mutants, and parent lines x parent lines. The plants developed poorly so that the results were not considered satisfactory. Indications were that certain mutants differed from the parents in their parasitic capabilities, but no definite conclusions could be drawn.

Hybridization and Genetic Studies

Jensen (24), in 1888, and Kellerman and Swingle (26), in 1890, showed that there were two smuts of barley, *Ustilago nuda* and *U. hordei.* Until recently these have been considered the only two smuts

of barley, altho Biedenkopf (3), in 1894, described another smut that he called *U. medians.* Nebel (33), in 1934, summarized the literature pertaining to intermediate barley smuts and, in addition, reported results of her own studies. The literature concerning floral infection is voluminous and will not be reviewed here since Tisdale and Tapke (40) have reviewed the literature to 1924.

Biedenkopf's intermediate smut resembled *U. nuda* in head type and chlamydospore wall markings, but sporidia were produced on the promycelium, as is true of *U. hordei.* According to Nebel (33), Herzberg, in 1895, mentioned *U. medians* Biedenkopf. Brefeld (4) examined this smut and states "Ich habe dies Material aus einer gütigen Sendung von Biedenkopf untersucht und möchte doch nicht glauben, dass man auf solche minimale Rauheit der Spore neue Arten gründen kann." No further mention of an intermediate smut is found in the literature until 1932. Possibly Brefeld's statement discouraged further study of *U. medians,* altho the intermediate types may have occurred commonly and may have been mistaken for *U. nuda* or *U. hordei.*

In 1932 Tapke (38) described *U. nigra* as a new loose smut of barley differing from *U. nuda* in ability to infect barley seedlings and in having darker spore masses. Tapke did not report the manner of germination of the chlamydospores. Nebel (32), in 1933, described a series of barley smuts intermediate between *U. nuda* and *U. hordei,* and in a later publication she reports further studies on the intermediate smuts. She considered her Type 4 similar to *U. medians* Biedenkopf and *U. nigra* Tapke. Vanderwalle (42) described another smut of barley that he called "charbon nu tardif." He regarded it as a hybrid between *U. hordei* and *U. nuda.*

In the fall of 1932 the writer became interested in what appeared to be loose smut of barley, collected by Dr. J. J. Christensen. The spore mass was dark chocolate brown to black, and the chlamydospores were echinulate and germinated by means of promycelia and sporidia. Hence, it obviously did not fit the description of *U. nuda* or *U. hordei* but seemed very similar to *U. medians.* Several sporidia were isolated and the resulting lines were studied.

The monosporidial lines were paired in all possible combinations and were found to be of two sexes. Combinations between monosporidial lines of *U. medians* and of *U. hordei* were then made. The compatible lines, on the basis of the presence of fusions, were then used in further studies.

Interspecific hybridization between smut fungi has been attempted by several investigators. The most extensive work was that done by Kniep (28), who reported that the following smuts fused with *U. hordei: U. bromivora, U. perennans* Rostr., *U. nuda* (Jens.) K. and S., and *U. tritici* (Pers.) Rostr.

Dickinson (11) was unable to obtain infection of oat seedlings with monosporidial lines of *U. hordei* or *U. levis* alone, but when sporidia of one sex of *U. hordei* were combined with those of an opposite sex of *U. levis* infection resulted. Apparently he did not determine whether chlamydospores were produced by this cross.

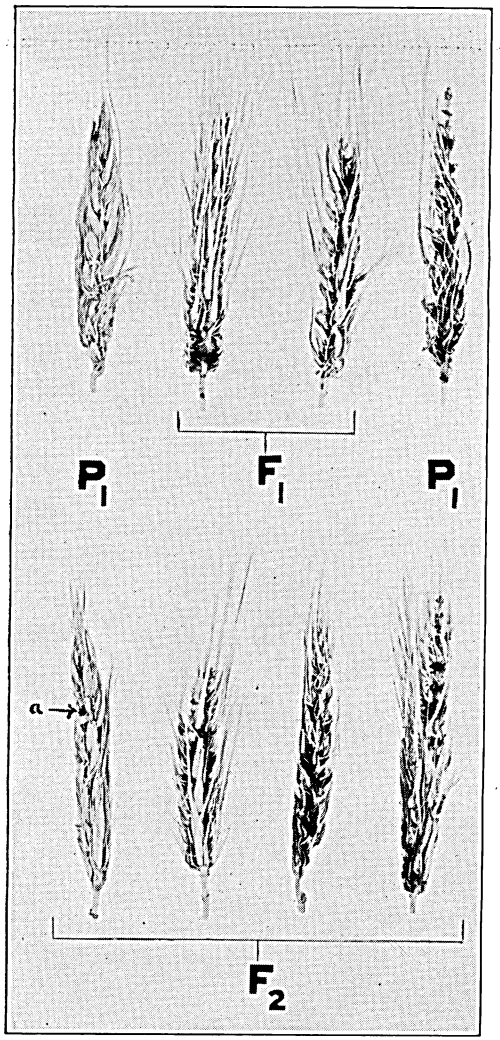

FIG. 4. SMUTTED HEAD TYPE OF TREBI BARLEY PRODUCED BY INTRA- AND INTERSPECIFIC SPORIDIAL COMBINATIONS

P_1 left = *U. hordei* 30 x 31
P_1 right = *U. medians* 24 x 25
F_1 = *U. hordei* 31 x *U. medians* 24, F_1
F_2 = *U. hordei* 31 x *U. medians* 24, F_2

Left to right smutted heads produced by the following f_1 monosporidial combinations: 49 x 48, 47 x 48, 49 x 50, and 47 x 50.

(a) Indicates where spikelet has been removed to show basal spikelet infection in heads smutted by the monosporidial combination 49 x 48.

Other interspecific crosses have been made in which hybrid chlamydospores were produced. Holton (22) and Hanna and Popp (19) obtained hybrid chlamydospores as a result of interspecific crosses between *U. levis* and *U. avenae*. The F_1 chlamydospores were echinulate like those of *U. avenae*. Flor (15) obtained hybrid chlamydospores by crossing lines of *Tilletia levis* Kühn and *Tilletia tritici* (Bjerk.) Wint. Rodenhiser (35) recently crossed haploid lines of *Sphacelotheca sorghi* (Lk.) Clint. with haploid lines of *S. cruenta* (Kühn) Potter and obtained viable hybrid chlamydospores. The sori produced were macroscopically like those of *S. cruenta*. More recently Tyler and Shumway (41) succeeded in crossing two genera, *Sphacelotheca sorghi* and *Sorosporium reilianum* (Kühn) McAlpine. The F_1 sori and chlamydospores appeared to be intermediate in type between those of the parents. The writer, in 1935, published a preliminary report on hybridization between *U. hordei* and *U. medians* (1).

Since sporidia of *U. hordei* appeared to fuse readily with sporidia of the opposite sex of *U. medians*, studies were made on the inheritance of various characters, such as smutted head type, spore wall markings, size of chlamydospores, and virulence on six barley varieties.

Seed of Trebi barley was inoculated with a number of intraspecific and interspecific sporidial combinations of *U. hordei* and *U. medians*. All combinations that produced typical fusions in culture also produced viable chlamydospores. In general, the F_1 head type of all the interspecific crosses was intermediate, tending somewhat toward the loose head type of *U. medians* (Fig. 4). Plants grown in the greenhouse from seed inoculated with the interspecific sporidial combinations produced F_1 smutted heads more nearly like those of *U. medians*. In fact, in one experiment the head type of the F_1 could not be distinguished from that of *U. medians* (See Fig. 5). In all of the interspecific crosses the F_1 chlamydospores were echinulate, like those of *U. medians*.

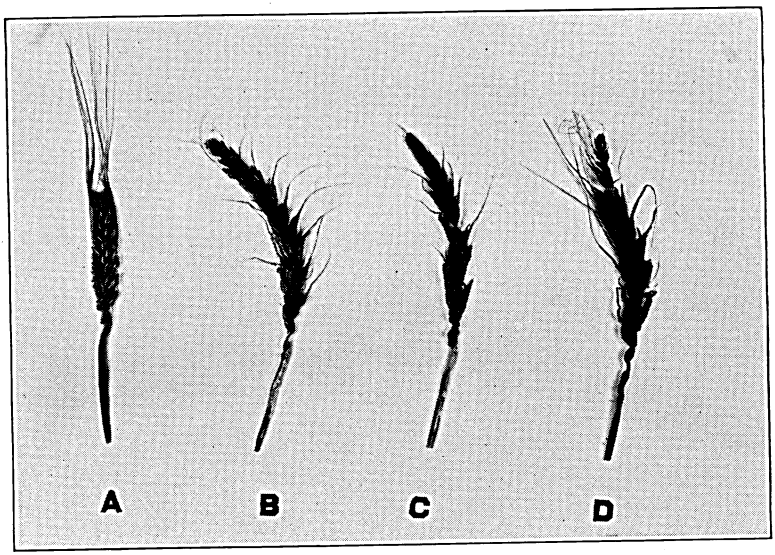

FIG. 5. SMUTTED HEAD TYPES OF MINN. NO. 474 BARLEY PRODUCED BY INTRA- AND INTERSPECIFIC MONOSPORIDIAL COMBINATIONS

A = *U. hordei* 30 x 31
B and C = *U. hordei* 31 x *U. medians* 24, F_1
D = *U. medians* 24 x 25

For detailed inheritance studies, monosporidial cultures 30 and 31 of *U. hordei* and 24 and 25 of *U. medians* were used. These combinations have been inbred for four generations and all characters have remained constant.

In studying the inheritance of head type and chlamydospore wall markings in the F_2, complete sets of sporidia from F_1 chlamydospores of *U. hordei* 31 x *U. medians* 24 were used. These sporidial lines were paired in all possible combinations, and seed of Trebi barley was inoculated with each combination. Only those combinations that fused in culture produced smutted heads. The chlamydospores used were designated K and N. The head type and chlamydospore markings of the F_2 are given in Table 7. In two crosses (N-2 x N-3 and N-3 x N-4)

it was impossible to determine the head type because the upper part of the culms and leaves were so heavily smutted that the heads did not emerge. In other cases it was difficult to determine definitely the head type, and these have been designated intermediate.

Table 7. F_2 Head Type and Chlamydospore Wall Character, Produced by f_1 Monosporidial Combinations of Ustilago hordei x U. medians on Trebi Barley

Monosporidial f_1 combinations	F_2 head type	F_2 spore wall	Monosporidial f_1 combinations	F_2 head type	F_2 spore wall
K-1 x K-2	L*	E	K-1 x N-2	I	S
K-1 x K-4	C	S	K-1 x N-4	I	S
K-2 x K-3	L	E	K-2 x N-1	L	E
K-3 x K-4	C	E	K-2 x N-3	L	E
N-1 x N-2	L	E	K-3 x N-2	C	E
N-1 x N-4	C	E	K-3 x N-4	L	E
N-2 x N-3	.....	E	K-4 x N-1	I	E
N-3 x N-4	.....	E	K-4 x N-3	L	E

* L = loose; C = compact; I = intermediate; E = echinulate; S = smooth.

The F_2 spore wall character of f_1 combinations from F_1 chlamydospore K segregated in the ratio of 3:1, with echinulation dominant. The same segregation in the F_2 occurred when f_1 lines from chlamydospore K were combined with those from N. The F_2 of f_1 combinations from chlamydospore N were all echinulate, 4:0. The explanation for this can readily be seen in Figure 6, which shows the segregation for sex and chlamydospore wall characters in the promycelium of F_1 chlamydospores as well as the recombination of the factors for chlamydospore

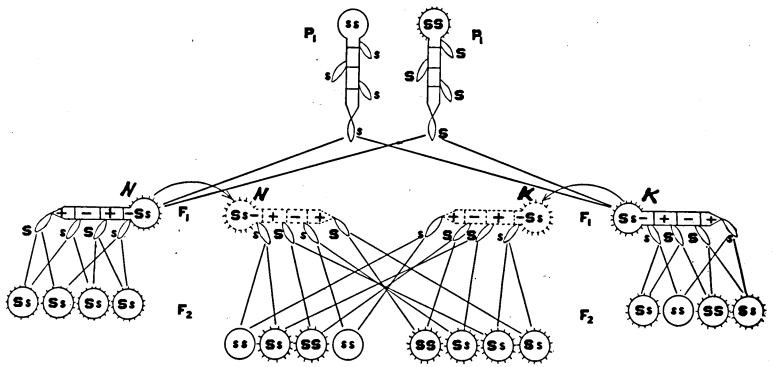

FIG. 6. SEGREGATION AND RECOMBINATION OF FACTORS FOR SEX AND SPORE WALL MARKINGS IN A CROSS OF *U. hordei* 31 x *U. medians* 24

wall characters in the resulting F_2 chlamydospores. Since echinulation is dominant and, on the basis of the F_2 of chlamydospore K and f_1 combinations of chlamydospores K and N, is dependent on a single factor difference, we may designate the factors for echinulation in *U. medians*

chlamydospores as SS. The factors for recessive or smooth chlamydospores may be designated ss. The resulting sporidia of *U. medians* will carry the factor S, those of *U. hordei* will carry the factor s. F_1 chlamydospores will carry one factor from each sporidium or Ss. In segregation in the promycelium of F_1 chlamydospores, each sporidium should receive the factor S or s. Judging from the F_2 chlamydospores this did occur for F_1 chlamydospore K, as shown in Figure 6. That is, of the four sporidial combinations one produced smooth chlamydospores and three produced echinulate chlamydospores. It will be noted that both + sporidia carried different factors for chlamydospore wall marking. The same was true for the — sporidia. The segregation for S and s could not be determined for F_1 chlamydospore N by sporidial combinations within N. However, it could be determined as the result of combinations of sporidia of chlamydospore N with those of chlamydospore K. In the case of chlamydospore N both — sporidia carried the recessive factor, s, while the + sporidia carried the dominant factor, S. Since chlamydospores were produced only when sporidial lines of opposite sex were combined, only F_2 chlamydospores with the factors Ss could be obtained from f_1 sporidial combinations from chlamydospore N. In addition to the parental types, compact head with smooth chlamydospores and loose head with echinulate chlamydospores, the F_2 consisted of the F_1 type, intermediate head with echinulate chlamydospores, and two new types, compact head with echinulate chlamydospores and intermediate head with smooth chlamydospores. These two new types have been found recently in nature by the writer and M. B. Moore.

Another character, the size of chlamydospores, was studied to determine whether the two species and their hybrids differed in this respect. The chlamydospore measurements, in microns, are given in Table 8 and each measurement represents the mean of 100. Chlamydospores produced by monosporidial cultures 30 x 31 of *U. hordei* had a mean width of 7.0 ± .03 microns and a mean length of 7.4 ± .03 microns. One hundred chlamydospores of *U. hordei,* collection 37, averaged 0.1 micron smaller than those produced by *U. hordei* 30 x 31, but this difference is not significant. The chlamydospores produced by *U. medians,* cultures 24 x 25, were 1.0 micron shorter and 1.1 micron narrower than the chlamydospores produced by *U. hordei* 30 x 31. These differences in size are significant and indicate the further advisability of considering *U. medians* a distinct species. The F_1 chlamydospores produced by *U. hordei* 31 x *U. medians* 24 were intermediate in size, closer to *U. hordei* than to *U. medians.* The range in chlamydospore size of the F_1 was greater than the range of either of the two parental combinations.

Table 8. The Size of Chlamydospores of Ustilago hordei, U. medians, and U. hordei 31 x U. medians 24

Source of chlamydospores	Mean length in microns	Mean width in microns
U. hordei 30 x 31	7.4 ± .03	7.0 ± .03
U. medians 24 x 25	6.4 ± .04	5.9 ± .03
U. hordei 31 x *U. medians* 24 F_1	7.1 ± .05	6.6 ± .04
U. hordei, collection 37	7.3 ± .04	6.9 ± .03

Table 9. Percentage of Smutted Heads, Head Type, and Chlamydospore Wall Character; Produced by Intra- and Interspecific Monosporidial Combinations of Ustilago hordei and Ustilago medians

Monosporidial combinations	Variety and per cent smut						Head type	Spore wall
	Velvet	Svansota	Peatland	Trebi	Odessa	Wis. No. 38		
U. hordei 30 x *U. hordei* 31	4	0	0	15	9	1	C*	S
U. medians 24 x *U. medians* 25	1	15	9	23	7	0	L	E
U. hordei 30 x *U. medians* 25, F_1	4	5	0	14	11	0	I	E
U. hordei 31 x *U. medians* 24, F_1	2	14	0	32	8	0	I	E
U. hordei 31 x *U. medians* 24, F_2								
47 x 48	1	14	9	33	12	0	I	E
47 x 50	12	13	9	31	15	0	L	E
49 x 48	2	13	10	15	9	0	.....	S
49 x 50	18	7	9	37	13	0	L	E

* L = loose; C = compact; I = intermediate; E = echinulate; S = smooth.

In the studies on inheritance of pathogenicity, the same parental cultures were used, but four f_1 monosporidial lines were selected from four F_1 chlamydospores. The term dicaryophyte is used to designate the pathogenic phase of the monosporidial combinations, since pathogenicity is actually the effect of the dicaryophyte in the plant, not of the haploid or true diploid stage. The same filial generation number has been applied to the dicaryophyte as to the diploid chlamydospores produced by it.

Six barley varieties were inoculated in the usual manner and sown at the same time in duplicate five-foot rows. The percentages of heads smutted by parental combinations and F_1 and F_2 dicaryophytes are given in Table 9. Head type and spore wall character are included.

The data were analysed by the analysis of variance method as developed by Fisher (14). From Table 10 it appears that there are significant differences between crosses and between varieties and in the interaction of crosses with varieties since the value of Z greatly exceeds the one per cent point. Taking twice the standard error of the difference between any two means ($2 \times 4.09 = 8.18$) as the minimum level of significance, it is evident that there are distinct differences in the reaction of the barley varieties to the different dicaryophytes.

Table 10. The Analysis of Variance of Percentage of Smutted Heads Produced by Intra- and Interspecific Monosporidial Combinations of Ustilago hordei and Ustilago medians

Variations due to	D.F.	Sum of squares	Variance	Z
Replications	1	27.26	.....	.....
Crosses	8	1,009.28	126.16	1.0111**
Varieties	5	5,800.00	1,160.00	2.1204**
Crosses x Varieties	40	1,615.68	40.39	0.4414**
Error	53	885.37	16.70	.....
Total	107	9,337.59	.....	.....

** Value of Z exceeds 1 per cent point.
S.E. of difference between two means = 4.09.

The intraspecific dicaryophyte, *U. hordei* 30 x 31, was significantly less pathogenic on Svansota, Peatland, and Trebi than the intraspecific dicaryophyte, *U. medians* 24 x 25. One F_1 dicaryophyte, *U. hordei* 30 x *U. medians* 25, was similar to *U. hordei* 30 x 31 on all of the varieties. The other F_1 dicaryophyte, *U. hordei* 31 x *U. medians* 24, was similar to *U. medians* 24 x 25 on Svansota, the same as *U. hordei* 30 x 31 on Peatland, but significantly more virulent on Trebi than either of the two parental combinations. The F_2 dicaryophytes from the latter interspecific F_1 were extremely interesting in their effects on Velvet, Svansota, Peatland, and Trebi. It will be noted that all of the F_2 dicaryophytes of *U. hordei* x *U. medians* were as virulent as the *U. medians* 24 x 25 parent on Peatland, altho the F_1 did not attack Peatland. Two F_2 interspecific dicaryophytes, 47 x 50 and 49 x 50, were decidedly more virulent on Velvet. On Svansota the virulence of three F_2 dicaryophytes was the same as that of *U. medians* 24 x 25 and of the F_1 interspecific dicaryophyte 31 x 24. The greater virulence of the dicaryophyte 31 x 24 on Trebi occurred in three of the F_2 dicaryophytes also, while one was similar in pathogenicity to the parent, *U. hordei* 30 x 31. None of the

F_2 dicaryophytes had the same virulence on all the varieties as either of the parental combinations or the F_1 dicaryophytes. This is of considerable practical importance, as it is obvious that interspecific hybridization could readily occur in nature and new strains of smut be produced that were more virulent on certain varieties than the parental strains.

The head type and spore wall character produced by the above intraspecific and interspecific crosses are given in Table 9 and a photograph of the head types is shown in Figure 4. The head type for the F_2, 49 x 48, could not be definitely determined because only the basal portion of the spikelet was smutted. This is a distinct type of smutted head not observed previously. In Figure 4 (a) a portion of a spikelet has been removed to show the smut in the basal portion. All of the heads affected were smutted in this manner.

Since the number of F_2 individuals studied was small, no genetic analysis of the characters studied could be made. A large number were used in a later study, but many of the inoculated barley varieties did not head because of the extremely dry weather. Despite the small number of crosses, indications are that the virulence of hybrids of *U. hordei* x *U. medians* may be entirely different from that of the parents, and increased virulence may result. The studies indicate also that virulence, head type, and chlamydospore wall character are probably not closely correlated.

In further hybridization studies monosporidial lines of *U. avenae* and *U. levis* were isolated by the writer and paired with various monosporidial lines of *U. hordei* and *U. medians*. In addition, some of the monosporidial lines of *U. avenae* and *U. levis* used by Holton (21) were made available to the writer. Fusions occurred between lines of opposite sex but not within haploid lines or between haploid lines previously shown to be of the same sex. Apparently there was no difference in the manner of fusions between intraspecific or interspecific combinations. Fusions occurred in about the same length of time in either case. Photomicrographs of fusions between sporidia of *U. hordei* and *U. levis* are shown in Figures 7 and 8. Other interspecific and intraspecific fusions were similar. See Figure 9.

In the intraspecific and interspecific combinations of *U. hordei* and *U. medians* aerial hyphae occurred rarely, except when lines of opposite sex were paired. Very often these aerial hyphae could be traced to the original fused sporidia. This was so striking that in all the combinations observed the character could be used as a criterion of compatibility. However, in certain combinations of sporidial lines of *U. levis* and *U. avenae* with *U. hordei* and *U. medians* it was noted that the aerial hyphae did not arise solely from fused sporidia but also from single sporidia. Observations on haploid lines of *U. avenae* and *U. levis* indicated that in these two species the aerial hyphae occurred rather frequently in some lines. Hence, altho the presence of aerial hyphae can be used as an indication of compatibility between haploid lines of *U. hordei* and *U. medians,* it is not reliable when certain sporidial lines of *U. levis* or *U. avenae* are used.

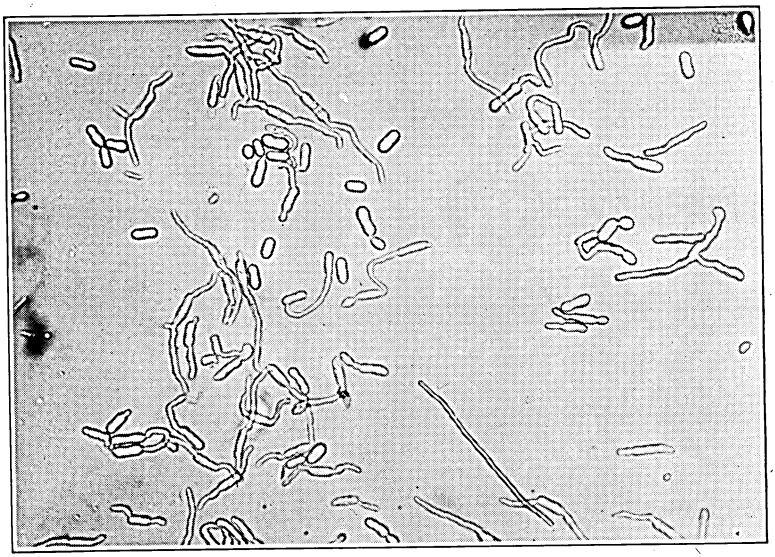

FIG. 7. PHOTOMICROGRAPH OF FUSIONS BETWEEN SPORIDIA OF *U. levis* AND SPORIDIA OF *U. hordei* (APPROX. 750X.)

Note dark aerial hypha in lower center.

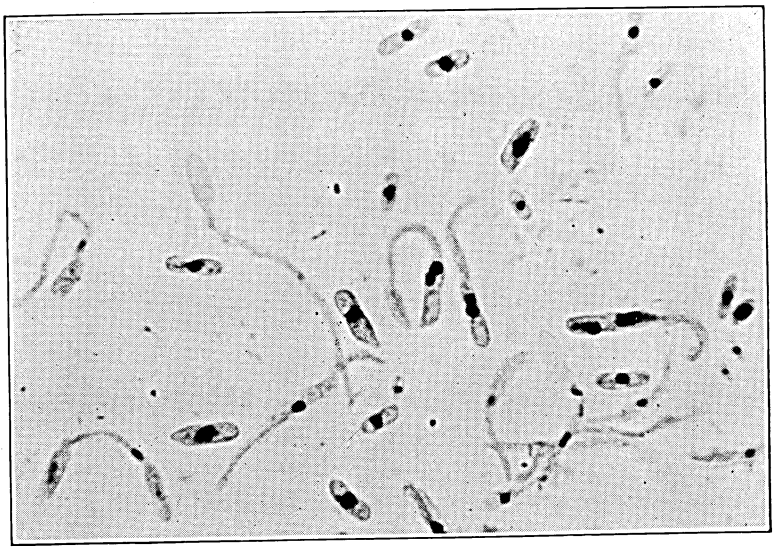

FIG. 8. PHOTOMICROGRAPH SHOWING NUCLEAR CONDITION OF FUSED SPORIDIA, *U. hordei* x *U. levis* (APPROX. 1600X.)

Center: Note U-shape structure in which the nucleus from the sporidium on left has passed into the sporidium on right.

Lower left: Note nucleus passing through fusion tube.

Figure 9 illustrates some of the fusion types observed in intraspecific and interspecific sporidial combinations. Actually, altho each figure represents either an intraspecific or interspecific combination, all of the figures could be used to represent the observed fusion types of any one intraspecific or interspecific combination. It will be noticed that Figures a, b, and c represent three different methods of fusion in the preliminary stages. In Figure a one sporidium has become active while the other has remained passive. This is the type of fusion observed by Holton (22) in *U. levis* and *U. avenae*. However, in Figure b both sporidia may be called active as each has produced a fusion tube. In Figure c the fusion pair is somewhat intermediate, one being more active than the other. After fusion has occurred, the U type of structure is commonly seen (Figures d and g). In many cases, within a few hours after fusion, the protoplasm tends to pass through the fusion tube into the other sporidium (Figures j, k, l, m, and o). The writer considers this a means by which the two nuclei are brought together. Soon after the protoplasm has passed into one or the other sporidium, a hypha usually is produced that tends to grow away from the agar into the air. Early stages in the production of such a hypha are shown in Figures m, n, and o. The later stages of the fusion pairs are shown in Figures q to u. The aerial hyphae (infection hyphae) are well developed, and the protoplasm has passed from the sporidia and fusion tube into the aerial hyphae.

Since the above interspecific fusions occurred between sporidial lines of opposite sex, it was considered probable that the oat smuts could readily hybridize with *U. hordei* and *U. medians* and produce viable chlamydospores. Consequently, in the spring of 1931, seeds of Trebi barley and Anthony oats were inoculated with several intraspecific and interspecific sporidial combinations. The compatible intraspecific combinations of *U. hordei* produced smutted heads on Trebi barley and the compatible intraspecific and interspecific combinations of *U. levis* and *U. avenae* produced smutted heads and viable chlamydospores on Anthony oats. The F_1 chlamydospores of the interspecific cross *U. levis* x *U. avenae* were echinulate, as described by Holton (20) and Hanna and Popp (19). None of the interspecific sporidial combinations of *U. hordei* x *U. levis* or *U. avenae* produced smutted heads. During the winter of 1931-32 further experiments were made, and the inoculated seed was sown in the greenhouse. Additional monosporidial lines were used from different chlamydospore collections, but similar results were obtained. In the summers of 1932 and 1933 the work was expanded, using numerous monosporidial lines; and in 1933 monosporidial lines of *U. medians,* as well as additional varieties of oats and barley, were included. In all, 11 varieties of barley and 6 of oats were inoculated with the various intraspecific and interspecific sporidial combinations. Again only the intraspecific and interspecific sporidial combinations of *U. hordei* and *U. medians* produced smutted heads on the barley varieties and only the intraspecific and interspecific combinations of *U. avenae* and *U. levis* produced smut on plants of the oat varieties.

Dickinson (11) reports the presence of smut hyphae in the young seedlings of oat plants grown from seeds inoculated with interspecific

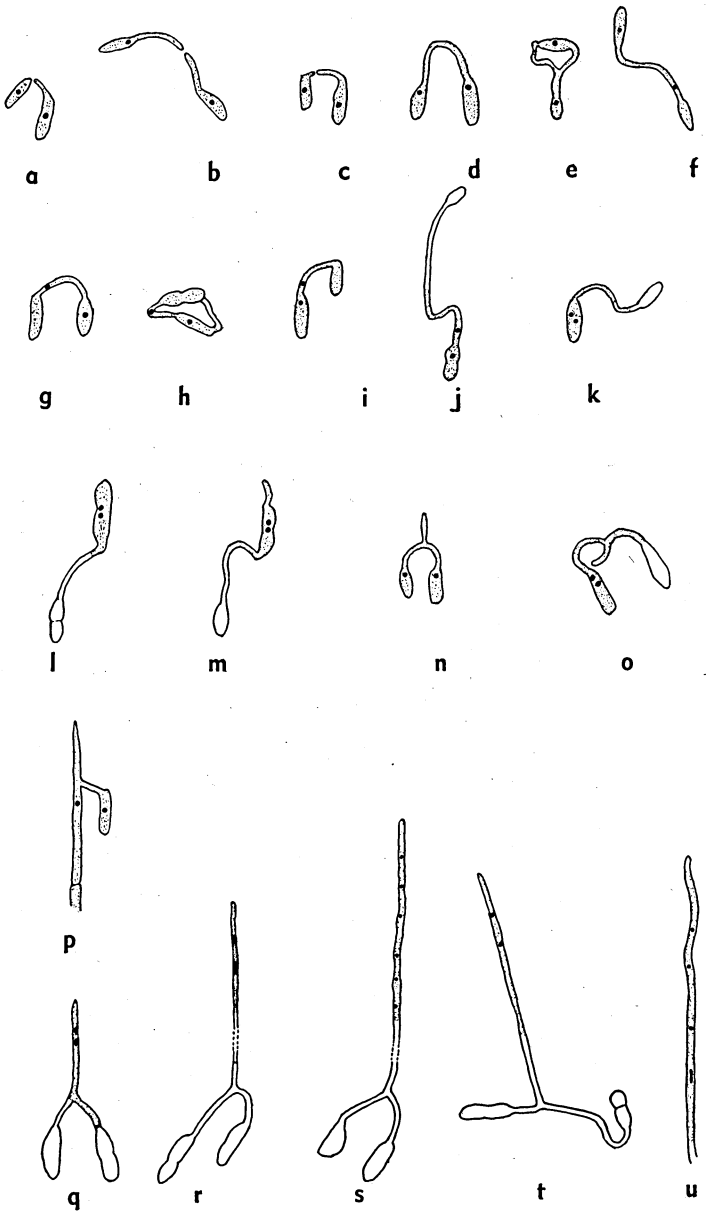

FIG. 9. INTRASPECIFIC AND INTERSPECIFIC FUSIONS SHOWING NUCLEAR CONDITION

a. *U. hordei* x *U. levis*
b. *U. hordei* x *U. hordei*
c. *U. hordei* x *U. levis*
d. *U. hordei* x *U. avenae*
e. *U. hordei* x *U. tritici*
f. *U. hordei* x *U. avenae*
g. *U. hordei* x *U. hordei*
h. *U. hordei* x *U. tritici*
i. *U. hordei* x *U. levis*
j. *U. hordei* x *U. tritici*
k. *U. hordei* x *U. avenae*
l. *U. hordei* x *U. tritici*
m. *U. hordei* x *U. tritici*
n. *U. hordei* x *U. levis*
o. *U. hordei* x *U. hordei*
p. *U. hordei* x *U. tritici*
q. *U. hordei* x *U. hordei*
r. *U. hordei* x *U. tritici*
s. *U. hordei* x *U. tritici*
t. *U. hordei* x *U. tritici*
u. *U. hordei* x *U. levis*

monosporidial combinations of *U. hordei* x *U. levis*. The writer examined several seedlings of barley and oats grown from seed inoculated with interspecific combinations of *U. hordei* and *U. medians* x *U. levis* and *U. avenae,* but was unable to find smut hyphae. Nevertheless, smut hyphae were abundant in five-day-old barley seedlings grown from seed inoculated with compatible intraspecific and interspecific combinations of *U. hordei* and *U. medians*. The same was true of oat seedlings derived from seed inoculated with compatible intraspecific and interspecific combinations of *U. levis* and *U. avenae*.

From the above experiments it appears that hybridization between the oat smuts and *U. hordei* and *U. medians* does not extend beyond sporidial fusion and development of the hyphae. From evidence now available, therefore, it seems unlikely that there is complete hybridization, with consequent production of new types of smut, between the smuts of barley and those of oats in nature. It is possible that, if additional monosporidial lines were used, certain combinations might be capable of infecting oats and barley. Possibly other varieties of oats and barley not used by the writer might be more susceptible to these interspecific combinations. However, failure to find hyphae of *U. hordei* x *U. levis* in the young seedlings, in addition to other negative results, makes it seem unlikely that complete hybridization occurs commonly, apparently because factors necessary for pathogenicity are lacking.

Kniep (27) observed fusions between sporidia of *U. hordei* and promycelial cells of *U. tritici*. The writer observed similar fusions and later observed fusions between haploid lines of *U. hordei* and *U. tritici,* (Fig. 9). The haploid lines of *U. tritici* available were isolated by Clyde Christensen and kindly given to the writer for use in hybridization studies. The method of obtaining these cultures, some of which were sporidial in nature, is given by Christensen (7).

The sex of the *U. tritici* haploid lines was determined by pairing them with several different haploid lines of *U. hordei* and *U. medians*. In this case fusions were very similar to the intraspecific and interspecific fusions described. See Figure 9.

Seed of Trebi barley was inoculated with compatible sporidial combinations of *U. hordei* x *U. hordei* and *U. hordei* x *U. tritici,* and was sown in the greenhouse and field plots. The *U. hordei* x *U. hordei* combinations produced smutted plants as usual, but no hyphae could be found in the seedlings and no smutted plants were produced from seeds inoculated with the *U. hordei* x *U. tritici* combinations.

It is evident that the occurrence of fusions between sporidia of different *Ustilago* species does not indicate that infection will result. This is only natural, since pathogenicity for a specific variety or crop plant is evidently dependent on certain combinations of genetic factors. If the suitable combinations can be brought together, then infection results following fusion of the sporidia.

It is obvious that one of the first suggestions as to the origin of *U. medians* would be that it possibly resulted from a cross between

U. hordei and *U. nuda.* To determine whether these two smuts could hybridize with the resultant production of a hybrid similar to *U. medians,* six haploid lines of *U. nuda* were isolated according to the method used by C. Christensen for *U. tritici* (7). The sex of these haploid lines was determined by pairing them with sporidial lines of opposite sex of *U. hordei.* Compatible combinations of haploid lines of *U. hordei* and *U. nuda* were grown in culture and seed of Trebi and Glabron barley was inoculated with these combinations by means of the partial vacuum method. The seed was sown in the spring of 1934, but, owing to the dry weather, only one-half of the plants headed. None of the plants from seed inoculated with the interspecific combinations of *U. hordei* x *U. nuda* produced smutted heads. A few smutted heads were produced on the plants of Trebi barley grown from seeds inoculated with the compatible *U. hordei* monosporidial combinations. The writer does not consider this sufficient evidence that hybridization does not occur, because under more favorable conditions the plants from seed inoculated with the interspecific *U. hordei* x *U. nuda* combination might have produced smutted heads. In addition, only seed was inoculated, whereas, since infection by *U. nuda* occurs at flowering time, floral inoculation also should be tried for the interspecific cross.

In 1935 M. B. Moore and the writer described an albino strain of barley smut with an intermediate head type and smooth chlamydospores (30). Sporidial fusions between monosporidial lines indicated two sex groups that were compatible with the two sex groups of *U. hordei* and *U. medians.* Since then seed of Trebi barley was inoculated with monosporidial combinations of *U. hordei* x albino, *U. medians* x albino, and albino x albino. The results indicate that the factor for albino or white-smutted head is recessive, since the F_1 smutted heads of albino x *U. hordei* and *U. medians* were dark while the albino x albino F_1 smutted heads were white, with an occasional dark-smutted head in four of the F_1 crosses.

Cytology

Fusions of sporidia with sporidia, promycelial cells with promycelial cells, and promycelial cells with sporidia have been observed by many investigators in the genus *Ustilago,* but the resulting nuclear phenomena have not been described in all cases. According to Kniep (28), Rawitscher found that when the promycelial cells of *U. nuda* or *U. tritici* fuse, a hypha develops from the fusion tube or from one or the other of the promycelial cells and the nuclei from the two cells pass into the hypha, thus initiating the dicaryophase. Hüttig (23) described various types of promycelial fusions and the cytologic phenomena in *U. hordei.*

The writer studied the nuclear phenomena associated with sporidial fusions, in an effort to determine if they might explain the ability or inability of intraspecific and interspecific haploid combinations to infect the host plants. Drawings of some of the fusions observed are shown in Figure 9. Only rarely was a sporidium observed, outside of fusion

pairs, that contained more than one nucleus. The rare cases may have been due to the division of a haploid nucleus prior to budding or germination. Very soon after fusion the nucleus of one sporidium passed into the other (Figures f, g, h, i). Figure h is a peculiar type of fusion seldom observed. It is evident that fusion was attempted, first or last, by means of the fusion tube on the right, but plasmogamy did not occur at this point. However, plasmogamy did take place by means of the fusion tube on the left, through which the nucleus is passing from one sporidium to the other. Figures k, l, m, and o represent later stages in which the nuclei of the fusion pairs are associated in one sporidium. At this stage, as a rule, the protoplasm has passed from one sporidium through the fusion tube into the sporidium containing the two nuclei. This passage of the protoplasm may be necessary to bring the two nuclei together, but certain fusion pairs were observed in which the nuclei were associated in one sporidium while both sporidia contained protoplasm. In these fusion pairs it is possible that the protoplasm may have carried the nucleus from one sporidium into the other and then have flowed back into the empty sporidium.

Soon after the association of the two nuclei in one sporidium a hypha is developed from one or the other of the sporidia, but more commonly from the fusion tube. Figures m and o show this early stage. Figure n shows a similar stage except that the nuclei are not associated. This type was rare, and it is possible that the growth from the fusion tube is not an infection hypha but the beginning of an ordinary hypha or sporidium. Figures q to u show the nuclear condition observed in the aerial or infection hyphae produced as a result of fusion. These hyphae probably are the ones that enter the host plant. The aerial hyphae did not stain as well as the sporidia, but a few were found in which the nuclear condition could be determined. In Figures q to u, the nuclei and the protoplasm have passed into the aerial hyphae. The lower nucleus in Figure r appears to be dividing. Figure u is a more advanced stage of the aerial hypha. In this case the fusion pair from which this hypha arose could not be definitely determined. Four nuclei were observed grouped in pairs, with the lower nucleus definitely dividing. Figure s is a more advanced stage not commonly observed. The six nuclei do not appear to be grouped in pairs but are spaced about equal distances apart.

As far as could be determined, there was very little difference in the type of fusion or the nuclear phenomena in intraspecific or interspecific fusions. Evidently the nuclear behavior in interspecific fusions is not the determining factor in pathogenicity. In addition to sporidial fusions, the writer studied the nuclear behavior associated with fusions of the promycelial cells of *U. hordei.*

When seed of barley was inoculated with chlamydospore collections by means of the partial vacuum method, it was noted that the chlamydospores germinated underneath the hull within 12 to 24 hours after the seed was sown. The manner of germination of these chlamydospores differed from that of chlamydospores on nutrient agar in that sporidia were not produced so abundantly. Many chlamydospores produced no sporidia but only a promycelium, the cells of which quickly fused. Similar fusion of promycelial cells of *U. hordei* has been observed by

several workers and is described by Hüttig (23). A large number of seeds were examined and it was noted that in nearly all cases, using different varieties and chlamydospore collections, this manner of germination was typical when chlamydospores were introduced between the hull and caryopsis. Consequently, it would seem that sporidial fusions do not always play an important role in the infection of the barley seedling.

The nuclear behavior associated with these fusions was investigated by taking thin strips of the hull to which germinating chlamydospores were attached, killing and staining them, then mounting them in glycerine. This method was not entirely satisfactory, but the nuclear condition of a few germinating chlamydospores could be determined. Evidently the dicaryophase can be initiated this way as well as by sporidial fusions.

Kolk (29) has given an excellent review of the literature concerning the cytological relationship of various smuts in the host plants. In addition, she has presented original investigations on the cytological relationship of *U. avenae* in its host.

The writer made a similar study of the development and nuclear condition of *U. hordei* in susceptible and resistant varieties of barley. These studies were not completed, but some of this material has been examined to determine the nuclear condition of the mycelium.

Seed of Trebi barley was inoculated with chlamydospores of *U. hordei,* collection 33. One-half-inch portions of the seedlings produced were killed, embedded in paraffin, sectioned, and stained with Haidenhain's iron-alum haematoxylin. Hyphal cells appeared predominantly binucleate. The cells of the hyphae were relatively long, and most sections did not show the full length of a hyphal cell with both septa well marked. However, in many sections portions of hyphal cells were observed in which two nuclei were relatively close together, altho more rarely there may be from one to several in a cell. The results agree with those of Kolk (29) for *U. avenae,* except that the binucleate condition appears to be more common in mycelium of *U. hordei* than in *U. avenae.*

As described by Kolk for *U. avenae,* there is usually a distinct widening of the smut hypha at the point where it penetrates the cell wall. After penetration, the hypha narrows again to its original diameter. Since very few cases of direct penetration have been studied, the writer is not able to conclude definitely that this is the usual method of penetration.

DISCUSSION AND CONCLUSIONS

The control of barley smuts, both by seed treatment and the use of resistant varieties, has long been complicated by the existence of two species of smut with different life histories. Further complications became apparent with the discovery of physiologic races of both of these species. In the present work, for example, it was found that many collections of *Ustilago hordei* could be differentiated on varieties commonly grown in Minnesota and neighboring states. While this demonstration of physiologic races in *Ustilago hordei* is not new, it is of value as indicating the probable importance of the phenomenon in a barley

improvement program. Obviously, when there are physiologic races within the two species, there is a likelihood that still others may arise as a result of hybridization between existing races.

The situation was still further complicated when it was discovered that there is a third species of barley smut, known as *Ustilago nigra* or *Ustilago medians*. While the existence of the third species would be of no practical importance if its appearance and life history were the same as that of either of the two long-known smuts, there are certain characteristics of the intermediate type that make it important. The difficulty is that it is impossible to know by the appearance of the smutted heads whether the life history is like that of *U. nuda* or that of *U. hordei*. The farmer does not know, therefore, whether his smut can be controlled by seed disinfection with chemicals, as in the case of covered smut, or only by means of the modified hot-water method, as is true of the true loose smut.

In the barley-growing areas of Minnesota there are many intermediate types of barley smut caused by what may be termed *Ustilago medians*. The only method of determining whether these intermediate types can be controlled by the modified hot-water treatment or by seed disinfection with chemicals is to examine the chlamydospores and make germination tests. Those that behave like the covered smut produce sporidia, whereas those that behave like loose smut do not. It would be desirable, therefore, to maintain a "smut determining" service for farmers, in order that they may know whether treating barley with chemicals would control the type of smut that occurs in their fields. It is evident from observations and experiments so far made that much of the smut that looks like loose smut actually behaves like covered smut and can therefore be controlled by disinfection with chemical dusts. Naturally, then, farmers can eliminate by a simple process of seed treatment much of the smut which looks like the true loose smut.

The existence of three species of barley smuts obviously makes the problem of developing resistant varieties more difficult, because the reaction of the same varieties may differ with respect to the three smuts. But the complexity of the situation is further increased because there are physiologic races of *Ustilago hordei*, many of which will hybridize freely with those of *U. medians*. Not only were various combinations of head type and morphologic characters of the spores obtained as a result of such crosses, but some of the resulting dicaryophytes differed also in pathogenicity.

The mere fact that many haploid lines of *Ustilago hordei* and *U. medians* are interfertile would not be important in the breeding problem were it not for the fact that it has been shown that some of the resulting dicaryophytes apparently are more virulent than any of the intraspecific combinations that were made. For example, some of these interspecific combinations appear to be more virulent on Velvet barley than many of the intraspecific ones. In recent years the writer has observed an increase in the amount of covered smut on Velvet barley in the field, which would indicate that similar interspecific hybrids may have arisen in nature also. There is clear-cut evidence that recombinations of factors for head type and chlamydospore wall markings, such as were made in

artificial crosses between *Ustilago hordei* and *U. medians,* take place also in the field, and this indicates that hybridization not only is possible but also actually is going on in nature.

Whether still wider hybridization is possible between the barley smuts and other cereal smuts has not been definitely demonstrated. It has been shown that several types of hybridization may occur in smut fungi; namely, (1) interbiotypic hybridization within a species, (2) hybridization between different species, (3) hybridization between different genera. In the present work the writer obtained conclusive proof of hybridization between biotypes within a species and of hybridization between species, i.e., *U. hordei* x *U. medians.* Attempts also were made to ascertain whether the barley smuts would hybridize with oat smuts. Fusion between sporidia of opposite sex of different species takes place readily, as previously pointed out by Kniep and Dickinson. However, in the writer's experiments the resulting dicaryophytes failed to infect either barley or oats. It is doubtful whether the term hybridization should be applied to a process restricted to the fusion between sporidia and the consequent association of haploid nuclei in the dicaryophytes. From the evidence now available, it would seem that the necessary factors for pathogenicity are not present in these interspecific dicaryophytes. The situation may be analogous to that in certain intervarietal crosses of *Puccinia graminis.* In this fungus, hybrids have been obtained between the *tritici* and *secalis* varieties. Aeciospores were produced on barberries, but they infected only barley and not rye or wheat. The explanation probably is that it requires two haploid nuclei of the *tritici* variety to infect wheat, two of the *secalis* variety to infect rye, but two of either or one of each enable the fungus to infect barley, as barley is susceptible to both *tritici* and *secalis* varieties. After all, fusion between haploid sporidia or promycelial cells is only the first step in hybridization. It is an act of copulation, not necessarily resulting in fertilization. Two haploid nuclei may become associated through plasmogamy, but the dicaryons may lack the necessary factors for parasitism, or the nuclei may lack entirely the ability to fuse. On the other hand, experiments of this type should be continued, as it is entirely possible that certain interspecific monosporidial combinations, such as those between smuts of oats and barley, may be pathogenic to certain varieties, either of oats or barley or both, and may produce fertile chlamydospores, i.e., true hybrids.

The partial vacuum method, based on that of Zade and others, which has been developed in this investigation makes hybridization studies relatively easy. It is particularly adapted to inoculation with combinations of haploid lines and is therefore particularly suitable for studies of this type.

SUMMARY

1. The partial vacuum method of barley seed inoculation with chlamydospores of *U. hordei* and *U. medians* was found to give much better infection than the dusting method. It was also the most satisfactory for inoculating barley or oat seeds with monosporidial combinations.

2. Collections of covered smut of barley differed from one another in their virulence on 11 varieties of barley. Twenty-seven of 28 col-

lections could be differentiated on 6 of the 11 varieties. Three collections could be further differentiated on Minnesota No. 474, grown in the greenhouse, on the basis of the type of smutted plant produced.

3. Factors for sex of *U. hordei* and *U. medians* segregated in the ratio of 2:2. Factors for cultural characters in *U. hordei* segregated in the ratios of 3:1, 2:2, and 0:4, and independently of the factors for sex. Variants were observed which differed culturally from the parent colonies.

4. *Ustilago hordei* hybridizes readily with *U. medians,* as evidenced by sporidial fusions and the production of viable chlamydospores. Chlamydospores in the F_1 are echinulate, and the smutted head type is intermediate, tending toward the loose smut type of the *U. medians.* The F_2 segregated for chlamydospore wall character and for head type altho these two characters did not appear to be linked. Combinations of head type and chlamydospore wall markings differing from either parent type were obtained in the F_2. Segregation for pathogenicity occurred in the F_2 dicaryophytes. Some F_2 dicaryophytes were more pathogenic than the parent dicaryophytes on certain varieties.

5. *Ustilago hordei* and *U. medians* hybridize with *U. avenae, U. levis,* and *U. tritici* to the extent of fusions and initiation of the dicaryophase. However, seed inoculations with these combinations did not produce smutted heads nor were smut hyphae found in the young seedlings. The meager experimental data on the hybridization of *U. hordei* and *U. medians* x *U. nuda* were negative as to production of smut mycelium or chlamydospores in the host plants.

6. The nuclear condition of the sporidial fusions appeared to differ very little in intraspecific and interspecific crosses. The dicaryophase was initiated soon after fusion when the two nuclei of a fusion pair became associated in the same sporidium.

7. When chlamydospores of *U. hordei* germinated underneath the hull of the seed, they typically produced a promycelium, the cells of which fused readily instead of producing sporidia. The nuclear condition of these fused promycelial cells is evidently similar to that of fused sporidia.

8. The mycelium of *U. hordei* is predominantly dicaryotic in the host plant. However, many hyphae were observed with one nucleus or more than two nuclei per cell.

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